NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 395

TOXICOLOGY AND CARCINOGENESIS STUDIES OF PROBENECID

(CAS NO. 57-66-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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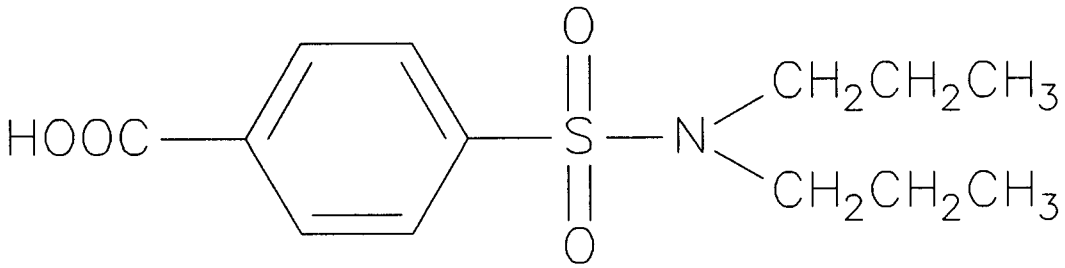
ABSTRACT



PROBENECID

CAS No. 57-66-9
Chemical Formula: $C_{13}H_{19}NO_4S$ Molecular Weight: 285.4

Synonyms: 4-[(Dipropylamino)sulfonyl]benzoic acid; *p*-(dipropylsulfamoyl)benzoic acid; *p*-(dipropylsulfamyl)benzoic acid
Trade Names: Benacen; Benemid; Benemide; Benn; Probalan; Probecid; Proben; Probenid; Robenecid; Uricocid

Probenecid is a white crystalline solid commonly used as a uricosuric agent in the treatment of gout. Because of its inhibitory effects on renal tubule transport processes, probenecid is also used as a therapeutic adjunct to enhance blood levels of penicillin and its action. Toxicology and carcinogenicity studies were conducted by administering probenecid (>99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex once daily, 5 days per week in 14-day, 13-week, and 2-year studies. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

14-Day Studies
Doses used in the 14-day studies for both rats and mice were 0, 200, 400, 800, 1,600, or 3,200 mg/kg. Of the animals receiving 3,200 mg/kg, all rats, all female mice, and two of five male mice died during the studies. No deaths occurred among the other dose groups. There was a significant reduction in body weight gain in male and female rats receiving 1,600 mg/kg and in female rats receiving 800 mg/kg. No gross lesions were attributed to probenecid administration in rats or mice of either sex.

13-Week Studies
Doses used in the 13-week studies were 0, 50, 100, 200, 400, or 800 mg/kg for rats and 0, 100, 200, 400, 800, or 1,600 mg/kg for mice. No rats died during the 13-week studies. In mice, 5 of 10 males and 3 of 10 females receiving 1,600 mg/kg and 1 of 10 males receiving 800 mg/kg died during the study. Significant reductions in body weight gain occurred in male rats administered 800 mg/kg, male mice administered 1,600 mg/kg, and female mice administered 800 or 1,600 mg/kg. All dose groups of male rats and all groups of female rats receiving 100 mg/kg or more showed significant increases in absolute and/or relative liver weights compared to control groups. This change was also seen in mice receiving 200 mg/kg and greater, except female mice in the 400 mg/kg group. No compound-related lesions occurred in rats or mice of either sex.

Based on compound-related deaths and suppression of body weight gains observed at higher doses in the 13-week studies, doses of 0, 100, and 400 mg/kg were used for the 2-year studies in rats and mice. These doses were administered once daily, 5 days a week for up to 103 weeks to groups of 50 males or 50 females of each species.

Body Weight and Survival in the 2-Year Studies

The mean body weight of high-dose female rats was 10% to 20% lower than that of controls throughout the studies. Mean body weights for all other dosed rats and for all dosed mice were similar to those of controls throughout the 2-year studies.

Survival of high-dose male rats and high-dose and low-dose male mice was significantly lower than that of controls. Survival rates after 2 years were: male rats—control, 37/50; 100 mg/kg, 34/50; 400 mg/kg, 22/50; female rats—24/50; 35/50; 19/50; male mice—38/50; 23/50; 24/50; female mice—32/49; 32/49; 32/50.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies

No chemical-related histopathologic toxic effects or increased incidence of tumors attributable to probenecid were observed in male or female rats receiving probenecid by corn oil gavage for up to 2 years. Mammary gland fibroadenomas and combined thyroid C-cell adenomas or carcinomas exhibited significant negative trends in female rats. These decreased tumor rates were associated with lower body weights. The incidence of adrenal medullary pheochromocytomas was significantly decreased in high-dose male rats. No compound-related increase in nonneoplastic lesions was observed in rats of either sex.

No compound-related neoplastic effects were observed in male mice. In high-dose female mice, there were significant increases in the incidences of hepatocellular adenomas (3/48; 2/49; 14/49), but there was no corresponding increase in carcinomas (2/48; 2/49; 3/49). Treatment-related increased incidences of ovarian abscesses in female mice were causally related to *Klebsiella* species infection rather than directly related to chemical administration.

Genetic Toxicology

Probenecid was not mutagenic in *Salmonella typhimurium* strain TA100, TA1535, TA1537, or TA98 with or without metabolic activation. In cytogenetic tests with Chinese hamster ovary cells, probenecid induced sister chromatid exchanges in the absence, but not in the presence of S9 activation. No induction of chromosomal aberrations was observed with or without S9.

Conclusions

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of probenecid for male or female F344/N rats receiving 100 or 400 mg/kg in corn oil. There was *no evidence of carcinogenic activity* of probenecid for male $B6C3F_1$ mice given 100 or 400 mg/kg probenecid in corn oil. There was *some evidence of carcinogenic activity* of probenecid for female $B6C3F_1$ mice based on an increased incidence of hepatocellular adenomas.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year and Genetic Toxicology Studies of Probenecid

Variable	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 100, or 400 mg/kg in corn oil by gavage 5 days a week at a volume of 5 mL/kg	0, 100, or 400 mg/kg in corn oil by gavage 5 days a week at a volume of 5 mL/kg	0, 100, or 400 mg/kg in corn oil by gavage 5 days a week at a volume of 10 mL/kg	0, 100, or 400 mg/kg in corn oil by gavage 5 days a week at a volume of 10 mL/kg
Body weights	No effect	Body weight depression in high dose	No effect	No effect
2-Year survival rates	37/50, 34/50, 22/50	24/50, 35/50, 19/50	38/50, 23/50, 24/50	32/49, 32/49, 32/50
Nonneoplastic effects	None attributed to probenecid	None attributed to probenecid	None attributed to probenecid	None attributed to probenecid
Neoplastic effects	None attributed to probenecid	None attributed to probenecid	None attributed to probenecid	Liver: hepatocellular adenomas (3/48, 2/49, 14/49)
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	Some evidence
Genetic toxicology				
Salmonella typhimurium				
Gene mutation:	Negative with and without S9 in strains TA100, TA1535, TA1537, and TA98			
Sister chromatid exchange				
Chinese hamster ovary cells *in vitro*:	Positive without S9			
Chromosomal aberrations				
Chinese hamster ovary cells *in vitro*:	Negative with and without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on probenecid on November 20, 1990 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS

On November 20, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of probenecid received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Committee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of probenecid by discussing the uses, experimental design, survival, body weight, and liver weight effects in rats and mice. He commented that the only compound-related lesions were hepatocellular tumors in female mice. The proposed conclusions were *no evidence of carcinogenic activity* for male or female F344/N rats or for male B6C3F$_1$ mice, and *some evidence of carcinogenic activity* for female B6C3F$_1$ mice.

Dr. Carlson, a principal reviewer, agreed with the conclusions. He commented on the statement that no chemical-related toxic effects were observed in male or female rats as being contradictory to statements in the results that "the moribund condition of these animals was presumed to be the result of chemical toxicity" and "these deaths were therefore presumed to be related to chemical toxicity."

Dr. Scala said the issue of moribund animals and the relationship of their condition to chemical toxicity needed to be clarified in the report.

Dr. Garman, the second principal reviewer, agreed with the conclusions. However, he questioned the combination of female mice hepatocellular adenomas and carcinomas in the summary table when the frequency of carcinoma was obviously not treatment related. Dr. Eustis, NIEHS, said the carcinomas would be separated out from the table.

Dr. Gold, the third principal reviewer, agreed with the conclusions. She suggested that the conclusions read "benign hepatocellular neoplasms," since the level of *some* rather than *clear evidence* in female mice was due to an increase in benign tumors only. Dr. Abdo said the conclusion would say "hepatocellular adenomas."

Dr. Carlson moved that the Technical Report on probenecid be accepted with the conclusions as written for male and female rats and male mice, *no evidence of carcinogenic activity*, and for female mice, *some evidence of carcinogenic activity*, with the last sentence being changed to emphasize that the conclusion was based on adenomas. Dr. Garman seconded the motion, which was accepted unanimously with eleven votes.